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# SURFACE TOPOGRAPHY OF *HASSTILESIA TRICOLOR* (TREMATODA: BRACHYLAIMIDAE) AS DEMONSTRATED BY SCANNING-ELECTRON MICROSCOPY[1]

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*Abstract.* SEM of *Hasstilesia tricolor*, a trematode parasite of rabbits, reveals retorse spines on the anterior, dorsal and ventral surfaces of the body. Spines are reduced on the suckers and are lacking at the posterior end. The spines penetrate the rabbits' mucosal cells and aid in holding the trematode in its tissue site between the intestinal villi.

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*Hasstilesia tricolor* Hall, 1916, a digenetic trematode, was present in 65% of 138 cottontail rabbits, *Sylvilagus floridanus mearnsii*, examined from 6 counties in central, northern Ohio. This trematode was the most prevalent platyhelminth parasite present. Trematodes of the Family Brachylaimidae may have either a spinous or nonspinous tegument, and some genera *Leuchochloridium*, *Postharmostomum* and *Brachylaima* may adhere tightly to the walls of their tissue sites by their large, acetabular suckers. Hensler (1959) reported that *Hasstilesia tricolor*, which does not have a large acetabular sucker (figure 4), was usually found free in the lumen of the rabbit intestine. Contrary to Hensler's observation, we found *H. tricolor* in the layer of mucus adhering to the lining of the intestine or between the intestinal villi with the spinous portion of the body in contact with the mucosa (figs. 9 and 10). This finding led us to examine the tegumentary spination of *H. tricolor* more carefully, utilizing scanning-electron microscopy.

## METHODS AND MATERIALS

Rabbits were trapped in wooden box traps (between April and September 1978) and transported alive to Columbus where they were necropsided. Short lengths of intestine processed for light microscopy were fixed in chilled, alcoholic Bouin's solution, embedded in paraffin, sectioned at 7 micrometers and stained with Harris' hematoxylin.

The surface topography of *H. tricolor* was examined with a Hitachi S 500 stereoscan microscope. Worms were cleaned in warm Ringer's solution, fixed in cold AFA solution, dehydrated, transferred to 100% acetone for 3 hr and dried in a Bomar SPC 900/EX critical-point dryer. Specimens were attached by No. 1481 silver paint to mounting stubs, placed in a Hummer III vacuum evaporator and sputter coated with gold to a thickness of 200 Å. During evaporation the specimens were rotated to insure complete coating. The SEM oscilloscope images were recorded on Polaroid PN-55 film.

## RESULTS AND DISCUSSION

Spines on the ventral surface of *H. tricolor* extended 80 to 87% of the body length from the anterior end (figure 1). The posterior 13 to 20% of the ventral surface is spineless, and there were no spines near the posterior, terminal, excretory pore (figure 7). Spines extended continuously over the anterior end and around the anterior lateral surfaces to the dorsal surface of the body (figs. 1 and 2). On the dorsal surface only the anterior 54 to 60% of the body tegument was spinous.

Spines on the ventral surface ranged from sharp to bluntly pointed (figure 6), but on the dorsal surface become more flattened and scale-like (figure 5). In the region of the oral and ventral suckers, spines were reduced, rounded and papillated with some extending onto the suckers (figs. 3 and 4). We identified specialized sensory papillae on the surface of suckers as found in some other trematodes. Spines surrounding the

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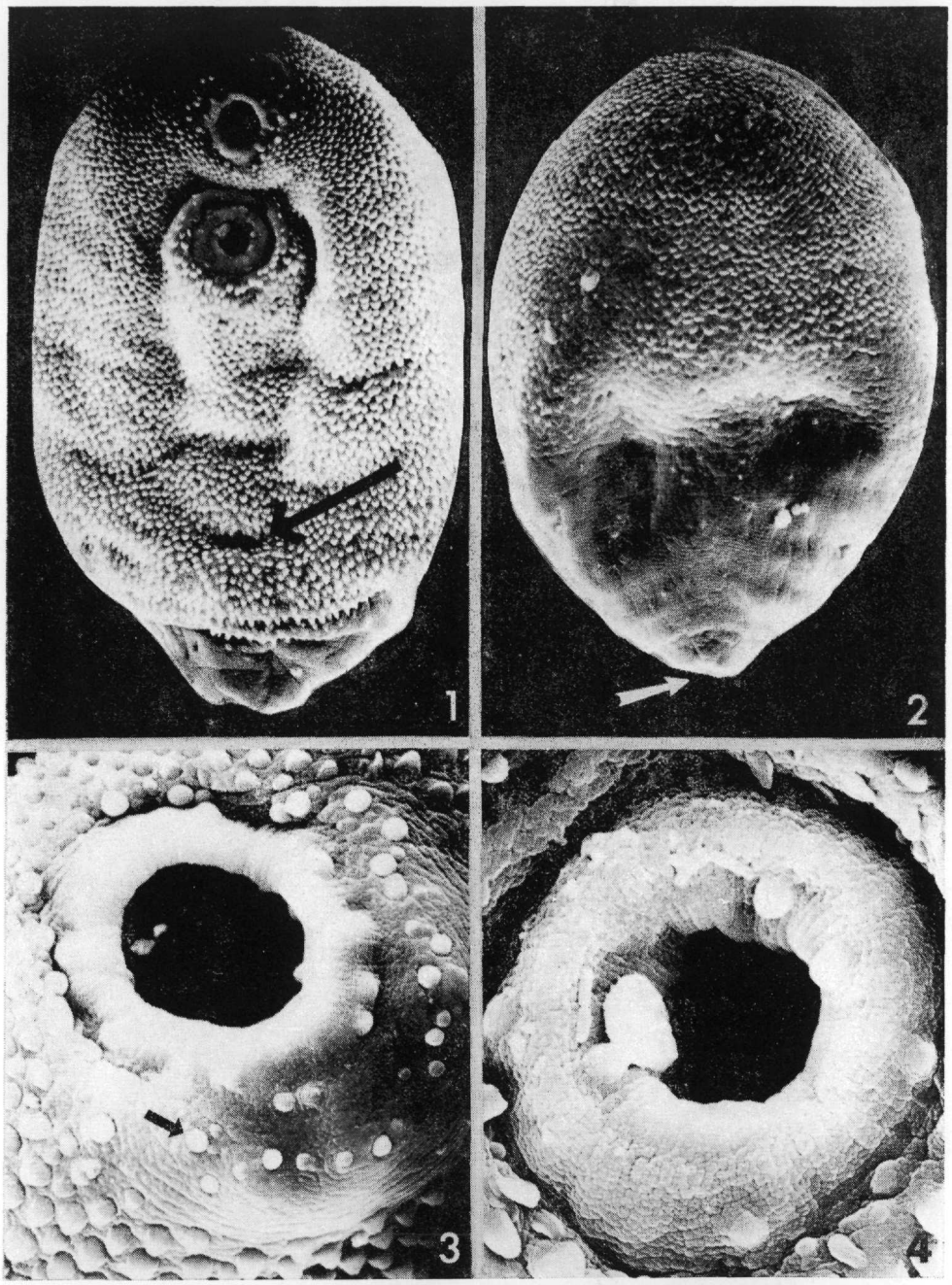
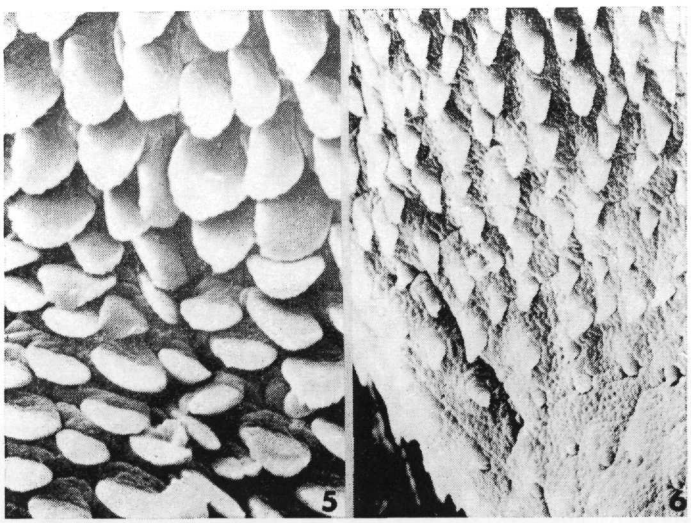
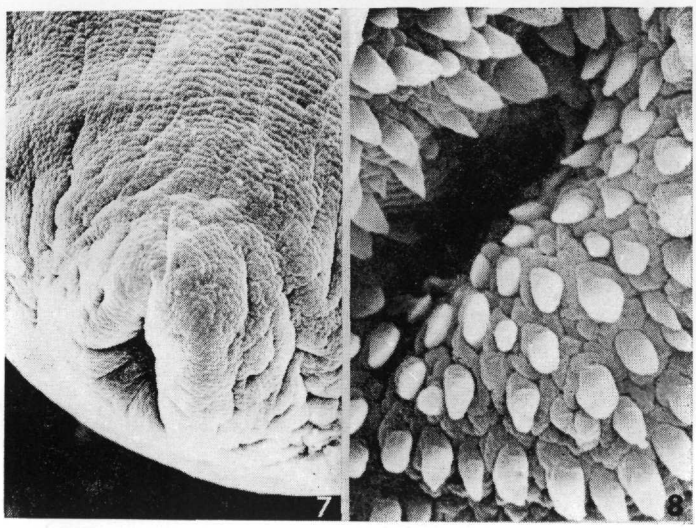



FIGURES 1–4. SEM of *Hasstilesia tricolor*. 1. Entire ventral surface, ×350; arrow marks the posterior genital pore. 2. Entire dorsal surface, ×300; arrow indicates the posterior end and area of the excretory pore. 3. Oral sucker, ×2000; arrow indicates papilla-like scale. 4. Ventral sucker, ×3000.



FIGURES 5–6. SEM of *Hasstilesia tricolor*. 5. Spines of dorsal surface, ×3000. 6. Spines of ventral surface, ×2000.

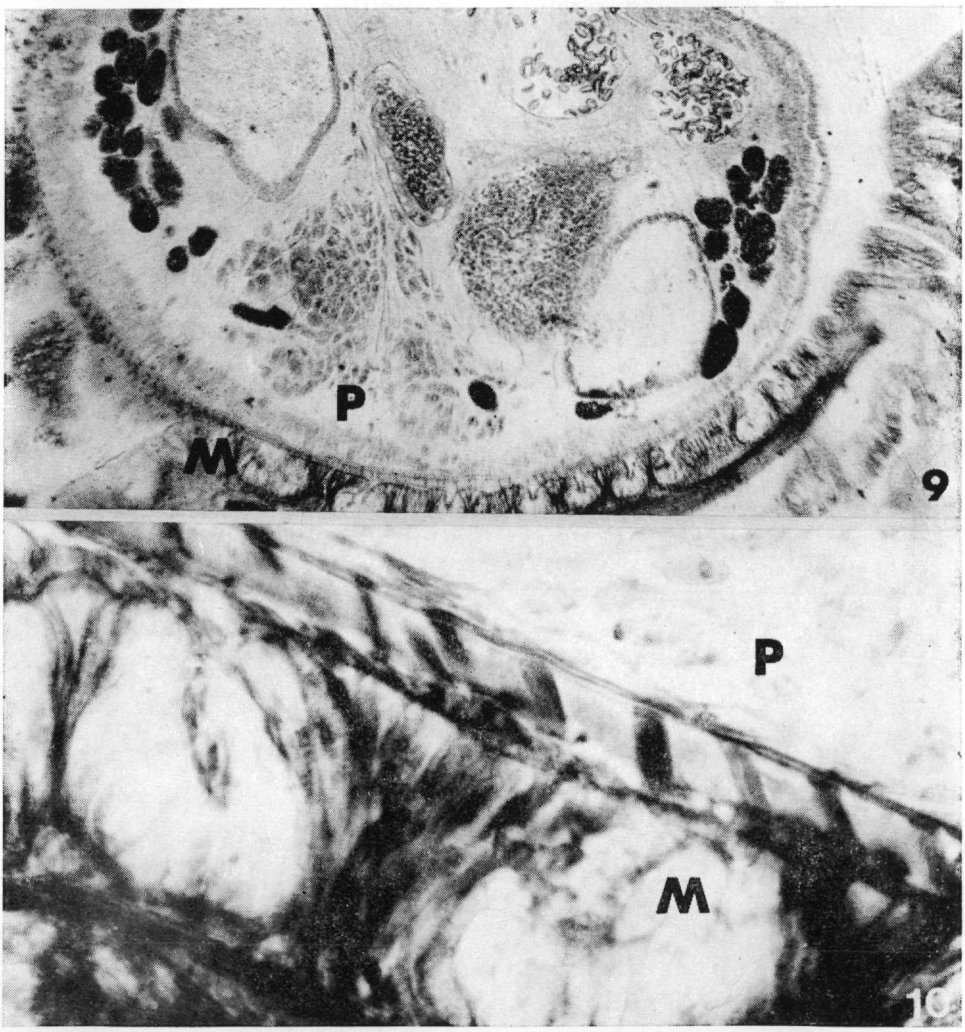


FIGURES 7–8. SEM of *Hasstilesia tricolor*. 7. Posterior excretory pore, ×1000. 8. Spines in region of genital pore, ×3000.

genital pore, on the posterior, ventral surface of the body, were sharp and not reduced (figure 8).

*Hasstilesia tricolor* occurred in greatest numbers in the anterior half of the duodenum of the rabbit hosts, but it spread throughout the ileum in intensely infected individuals.

It is our observation that *Hasstilesia tricolor* usually occurs in the mucus between the intestinal villi of the rabbit definitive host. It probably moves into place using its suckers, but remains in place primarily because of its retorse spines which protude into the microvilli of the mucosal cells (figure 10). The anterior end of the trematode is directed into the crypts and the posterior end is exposed to the intestinal lumen (figure 9). Eggs pass out of the genital pore in the posterior one fourth of the trematode's body and subsequently mix with the feces of the rabbit host. Rowan (1955) discovered that the eggs of *H. tricolor*




FIGURES 9–10. Light photomicrographs of cross-section of duodenum of rabbit with *H. tricolor* in position. 9. P indicates the parasite, M the mucosa of the rabbit host, ×50. 10. Spines of the parasite (P) protruding into mucosal cells (M), ×430.

were ingested by the small land snail, *Vertigo ventricosa elator* Sterki. He demonstrated that metacercariae develop from cercariae inside tubular sporocysts and never leave the snail intermediate host. Rabbits became infected when they accidentally ingest the small, infected snails. Taft (1961) records the genus *Vertigo* from these counties except for Marion and Seneca. The six Ohio counties where our rabbits were collected are Franklin, Delaware, Marion, Seneca, Ottawa and Erie. These counties all have limestone or dolomite substrates, and shell bearing land snails are plentiful.

## LITERATURE CITED

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